THE ENVELOPE OF THE RED CORPUSCLE AND ITS ROLE IN HÆMOLYSIS AND AGGLUTINATION.

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One of the most important fields of medical research at present is that which relates to hæmolysis. The brilliant theories of Ehrlich have served to explain a large number of facts bearing on the subjects of hæmolysis and immunity. But Ehrlich's work, while it concerns itself almost exclusively with the various properties and modes of action of the biological lakers, does not sufficiently take into account the chemical, physical, and histological changes which take place in the corpuscles themselves, when these are subjected to the action of hæmolytic agents. The importance of determining as far as possible these alterations is very clear, and it is evident that only after we have an intimate knowledge of the structure of the blood corpuscles can the nature of these changes be understood.

Two theories have been advanced concerning the structure of the red blood corpuscle. According to Schäfer and others, the corpuscle is merely a vesicle and consists of an envelope which contains a fluid mass. The other—proposed by Rollet and modified by Hamburger—assumes that the corpuscles consist of a network or stroma in whose meshes are found a fluid or semifluid mass composed of hæmoglobin and electrolytes.[1]

The first hypothesis is held by very few at the present date, while the latter—which has the support of many experimental facts—is endorsed by the majority of hæmatologists. Hamburger ably discusses the latter theory and gives good reasons for its acceptance. One argument which seems particularly convincing may be quoted here: "Osmotic experiments have shown that the amount of water which a corpuscle can imbibe and give out—*i. e.*, the amount of swelling and shrinkage which it is capable of— is less than would be the case if the corpuscle were simply a vesicle. Therefore, the interior of the corpuscle must be filled up with some protoplasmic substance—a stroma or network in whose meshes is contained the water-attracting fluid of the corpuscle."[2]

As Dr. G. N. Stewart has pointed out, the presence of an envelope or limiting layer about the corpuscles seems an almost necessary assumption.[3] It is well known that there is a constant interchange of substances between the corpuscles and surrounding medium; while the metabolic products and salts within the corpuscles make their way out, these are replaced by the requisite serum constituents.

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[1] Hamburger, Osmotischer, Druck und Ionenlehre, p. 171.

[2] Ibid.

[3] Journal of Physiology, 1899, vol. xxiv. p. 211.

On the other hand the corpuscles keep out bodies inimical to themselves. Both of these processes which are continually going on together, and which require a selective ability on the part of the corpuscles, could hardly take place were they not provided with envelopes which permit of the entrance of some substances and the exclusion of others.

It is the principal object of this paper to adduce evidence of the existence of such an envelope and to point out the role which it plays in hæmolysis and agglutination.

The presence of an envelope may be demonstrated by histological, chemical, and physical methods.

HISTOLOGICAL PROOF. The most obvious way of demonstrating the limiting membrane of a cell is by means of differential staining, but this has proved itself to be an especially difficult method in the case of blood corpuscles. I have been able to find in the literature only one reference to a staining reaction, whereby an envelope is said to be made visible in the red blood corpuscles.

H. Deetjen[1] stains corpuscles dried on a slide and heated to a certain temperature, with gentian violet; the envelopes, according to him, are thus easily made apparent.

Dr. G. N. Stewart has published some histological plates in which is given convincing evidence of the presence of an envelope in the necturus corpuscle.[2]

In the case of some cells the envelope may be clearly seen if it be mechanically separated from the underlying intracellular substance. By this method, for instance, the sarcolemma of a muscle fibre is rendered visible.

Through a peculiar reaction which takes place when hydroxylamine hydrochlorate is added to blood, I think it is possible to produce the above-mentioned separation of envelope and cell contents in the case of mammalian red corpuscles.[3]

In this reaction bubbles of nitrogen gas are formed at the periphery of the corpuscles. These bubbles are lined externally by a delicate, apparently hæmoglobin-free membrane (Fig. 1, *a*), and internally by hæmoglobin (*b*).

The membrane is hardly stained by any of those dyes which stain the hæmoglobin in the rest of the corpuscles very deeply. This indicates that it does not contain hæmoglobin. It appears colorless in the unstained corpuscle. This might be because the color of a very thin layer of hæmoglobin might escape detection. Only by observing the corpuscles during the formative stage of the bubbles is it possible to make out that they are not formed within the hæmoglobin at all, but that the latter merely forms their inner boundary.

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[1] Virchow's Archiv f. experimentelle Pathologie, 1901, vol. clxv. p. 282.

[2] American Journal of Physiology, 1902, vol. viii. p. 103.

[3] Peskind. Ibid., 1903, vol. viii. p. 424.

About ten minutes after the reagent and blood are mixed, the first sign of the intracorpuscular bubble formation becomes evident; it consists in a flattening or depression of the hæmoglobin on the periphery of a corpuscle, the outer lining of the bubble being too close to the hæmoglobin at this stage to be visible (Fig. 2).

The next stage is represented by Fig. 3, and shows some separation between the surface layer of the corpuscle (*a*) and the hæmoglobin.

The completed bubble appears in Fig. 4, the outer lining (*a*) often projecting considerably above the surface.

The most striking part of the reaction lies in the depression of the hæmoglobin from the surface of the corpuscle in the first stage. It demonstrates that the accumulation of gas takes place between an enveloping layer and the hæmoglobin, causing the repulsion of the latter from the surface of the corpuscle toward the centre and, at the same time, separating the envelope from the hæmoglobin underneath.

FIG. 1.

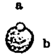

FIG. 2.

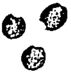

FIG. 3.

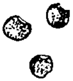

FIG. 4.

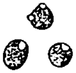

FIG. 5.

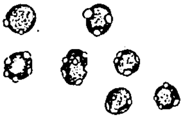

FIG. 6.

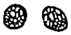

FIG 7.

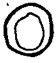

FIG. 8.

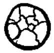

FIG. 9.

Although gas is formed immediately on mixing the blood and reagent, yet about ten minutes elapse before bubbles are formed inside the corpuscles. This could be explained on the hypothesis that the envelope must be hardened to a certain extent before it will be sufficiently unyielding to retain the bubbles within the corpuscles.

Efforts were unsuccessfully made to produce a differential stain of the membrane-like film covering the bubbles.

Several facts will be observed in this reaction:

1. That the bubbles are lined by a delicate and very thin membrane. This, together with the observations made on the evolution

of the bubbles, renders it almost certain that the membrane lining the bubbles is the envelope of the corpuscles.

2. That the bubbles are all of small size and sharply circumscribed. This speaks very strongly for the presence of a stroma whose meshes are continuous with trabeculæ derived from the envelope; for, if the envelope were a differentiated membrane and not connected to the intracellular substance, then we would expect that the gas formed in the reaction as it collects at the periphery of the corpuscles would produce a diffuse and extensive stripping of the envelope from the contents underneath, and would not form localized accumulations or bubbles. (See Fig. 7.)

But if the envelope is part of the stroma which forms the boundary of the corpuscles, then we can readily understand how the gas accumulation would be limited to the space between the trabeculæ, thus giving rise to bubble formation. (See Fig. 8.)

CHEMICAL EVIDENCE. We come next to the consideration of such chemical facts as point to the presence of an envelope.

In a recent paper I have described a reaction between blood corpuscles on the one hand, and acids and acid salts on the other.[1] When a mere trace of most acids and acid salts is added to a suspension of blood corpuscles, the latter are immediately agglutinated and precipitated. If the corpuscles be laked, then the stromata are precipitated by the same reagents. This reaction is apparently due to the combination of the nucleoproteid of the surface layer of the corpuscles with the reagents. The reasons for this view are given elsewhere.[2]

An interesting observation in connection with this reaction throws light on the subject under consideration.

We know that all the precipitating agents—*i. e.*, acids and acid salts in dilute solution—convert oxyhæmoglobin first into methæmoglobin and then into acid hæmatin. Now if the hæmoglobin in the intact corpuscles were present in the surface layer, then we should expect it to be rapidly altered by the precipitating agents into methæmoglobin. But it was found that the hæmoglobin contained in the corpuscles was not altered in the least if the exact amount of reagent necessary to precipitate the latter was employed. Even if a moderate excess of reagent were employed in precipitating the corpuscles no methæmoglobin band appeared for five or ten minutes. However, if we lake the corpuscles first—*e. g.*, by sapotoxin or water—and then add the same amount of reagent, it will be observed that part of the hæmoglobin is instantly converted into methæmoglobin and this conversion proceeds rapidly until complete.

What is it that temporarily shields the hæmoglobin in the intact corpuscles from the action of the reagents?.

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[1] American Journal of Physiology, 1903, vol. viii. p. 404.

[2] Ibid., p. 420.

Is this protection due to the fact that an intervening layer on the periphery of the corpuscle must be penetrated before the hæmoglobin can be reached, or is this conversion delayed by some chemical constituent of the corpuscles which combines with the reagent as rapidly as it enters? Such a chemical protection could be afforded to the hæmoglobin by the alkali of the corpuscles, by the stroma substance, or through the firm combination of the hæmoglobin with the stroma.

It can be shown that neither the alkali nor the stroma substance shields the hæmoglobin from the action of the reagents, since after laking the presence of stromata and liberated alkalies protects the oxyhæmoglobin but slightly and not at all if excess of reagent be present.[1]

Moreover there are many facts which suggest that the hæmoglobin compound within the corpuscle is a weak one and is easily split up.

Seeing that the temporary immunity of the hæmoglobin cannot be explained on chemical grounds, we are forced to adopt the view that there is something in the *structure* of the corpuscles, some mechanical or physicochemical barrier, which shields the hæmoglobin from the immediate action of the reagents. In other words, the hæmoglobin is protected by an envelope.

The laking of blood corpuscles by ether affords additional evidence of the existence of an envelope. It has been generally assumed that ether laking is due to the removal from the corpuscles of some or all of their cholesterin and lecithin.

This I have attempted to prove along the following lines:[2] First I determined how much ether is necessary to exactly lake 5 c.c. of blood. Then I extracted the corpuscles derived from 5 c.c. of blood with ether. The extract consisting of cholesterin and lecithin was suspended in 5 c.c. of serum or saline. Then ether was added cautiously to this suspension until the cholesterin and lecithin were perfectly dissolved and the amount of ether used noted.

It was found that the amount of ether necessary to dissolve the cholesterin and lecithin contained in the corpuscles of 5 c.c. of blood and suspended in 5 c.c. of saline or serum was about one-half again as much as was required to lake 5 c.c. of blood. So that the amount of ether required to lake blood bears a definite relation to the quantity of ether necessary to dissolve the cholesterin and lecithin of its corpuscles.

It appears from the above experiments that only a certain proportion of the cholesterin and lecithin is dissolved in ether laking, and this is probably the part that exists in the peripheral layer of the corpuscle. The cholesterin and the lecithin which remains undissolved during the laking in all probability remains in the interior of the corpuscle—*i. e.*, the stroma.

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[1] American Journal of Physiology, 1903, vol. iii. p. 422.

[2] These experiments will be published elsewhere in detail. Here I assume, in accordance with the general view, that cholesterin and lecithin are the only bodies dissolved out of blood corpuscles by ether. If, as some investigators believe, other ether-soluble substances exist, then my experiment shows that laking can be caused by the removal of other substances than cholesterin.

That the removal of lecithin alone from the envelope gives rise to laking can, I think, be readily demonstrated by the use of ether saturated with cholesterin.

If, as we have reason to believe, the cholesterin in the blood corpuscles has the same properties as the ordinary commercial cholesterin (Merck's was used in my experiments), then it would not be dissolved out from the corpuscles by ether which has been saturated with the commercial cholesterin. I found by experiment that a given amount of blood was laked by practically the same quantity of cholesterin-saturated ether as of pure ether, and, moreover, that the time required in the laking was the same in both instances.

The ether which was saturated with cholesterin laked blood just as though no cholesterin were dissolved in it. Here, I believe, we have good evidence to show that the removal of lecithin alone is capable of producing laking of the corpuscles.

PHYSICAL PROOF. Dr. G. N. Stewart has made an extensive research on the conductivity of blood corpuscles. As was mentioned previously, the necessity for assuming the presence of an envelope in all animal cells became clear to him early in his experiments, and realizing this he set about to determine what changes could be produced in the envelope of the red blood corpuscles by various laking agents, using them as convenient test-objects. Unfixed corpuscles being unsuitable for various reasons, he employed formaldehyde-hardened corpuscles. Sapotoxin and sodium taurocholate when added to a suspension of formaldehyde-hardened corpuscles produced the surprising effect of greatly increasing the conductivity of the latter.

"Unhardened blood corpuscles or formaldehyde-hardened corpuscles not treated with saponin or sapotoxin permit NH_4Cl to pass much more readily into them than NaCl, and there is evidence that some of the NH_4Cl is bound by substances in the corpuscles or at any rate does not pass as freely out of them as it passes into them. After the formaldehyde-hardened corpuscles have been acted on by sapotoxin they seem to exhibit little if any power of binding either NH_4Cl or NaCl, and the ions both of NaCl and NH_4Cl wander easily through the corpuscles."[1]

It will be seen from the above that there is beyond doubt a great change in the surface layer or envelope of the corpuscles after they are acted upon by the sapotoxin.

For the purpose of determining what constituents of the formaldehyde-hardened corpuscles are acted upon by sapotoxin the corpuscles were extracted with ether. The solvent took up a considerable quantity of cholesterin and lecithin. The ether-extracted corpuscles were carefully washed many times with salt solution and then suspended in a known volume of salt solution. For this suspension, Dr. Stewart found "the conductivity markedly greater than for a suspension of the unextracted formaldehyde corpuscles containing approximately the same proportions of corpuscles and salt solution. Sapotoxin caused no change in the conductivity of the extracted corpuscles. This renders it highly probable that sapotoxin increases the permeability of the formaldehyde corpuscles by action on the substance soluble in ether."

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[1] G. N. Stewart, Journal of Medical Research, No. 1, vol. viii. p. 270.

It can readily be shown that when a suspension of cholesterin is shaken up with sapotoxin the latter is removed by the cholesterin or rendered inert so that the liquid obtained after filtering off the cholesterin has no laking power. Apparently a definite amount of cholesterin neutralizes a certain amount of sapotoxin. I found, moreover, that formaldehyde-hardened corpuscles will also remove sapotoxin completely from a solution in twenty-four hours. So that changes in permeability produced by adding sapotoxin to formaldehyde-hardened corpuscles is clearly due to a combination of the sapotoxin with the cholesterin contained in the surface layers of the corpuscles.

While it seemed most likely to Dr. Stewart that sapotoxin exerted an action on unfixed corpuscles similar to that produced on formaldehyde corpuscles—and he gave evidence to that effect—he was unable at the time of the first research to prove this. The difficulty lay in the rapidity with which the sapotoxin causes laking. During the course of some experiments on hæmolytic agents, I made the observation that cold retards sapotoxin laking for a considerable period. This provided the means of proving the above-mentioned surmise, and to this effect a series of experiments, in some of which I assisted, were carried out. The conclusion drawn from the research are taken from Dr. Stewart's paper.[1]

1. At 0° C. the laking action of sapotoxin is much retarded, and it can be shown that before any hæmoglobin has been liberated the conductivity of the blood is increased, and presumably owing to the increase in the permeability of the corpuscles to electrolytes. At this stage sapotoxin has been fixed by the corpuscles.

2. Both at 0° C. and at ordinary temperature a dose of sapotoxin just sufficient to cause liberation of the hæmoglobin causes the discharge of a small proportion of the electrolytes of the corpuscles; when the dose is increased the electrolytes are discharged.

3. It seems permissible to divide the action on the corpuscles in three stages:

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[1] American Journal of Physiology, No. 2, vol. ix. p. 96.

(*a*) An action on the envelope, which does not necessarily nor immediately lead to the liberation of the hæmoglobin.

(*b*) An action on the hæmoglobin or the stroma which causes a discharge of the pigment.

(*c*) An action on the stroma leading to the setting free of electrolytes.

RESISTANCE OF BLOOD CORPUSCLES. The so-called "resistance of blood corpuscles" affords good evidence of the existence of an envelope. "It is well known that in certain physiological states and in disease laking begins to take place in a more concentrated salt solution than is required to lake normal corpuscles. This could be due to one of several causes—either that the stroma contents of the corpuscles in disease have a greater power of absorbing water or that they possess a greater volume—or that the envelope of the corpuscles has been so altered by the disease that, when it is stretched to a certain degree, it is less able to prevent the escape of hæmoglobin than would the envelope of a normal corpuscle. That the latter is in all probability the correct explanation may be seen from the action of hæmolytic and certain bacterial toxins on blood corpuscles.

"These poisons, in very small doses, alter the corpuscles in such a manner that they cannot bear such small salt concentrations as in the normal state. This change is found to be due neither to a modification of the water-absorbing power of the corpuscular contents nor to an increase of the intraglobular volume. So that we are forced to the assumption that the hæmolytic sera give rise to a diminished resistance of the protoplasmic envelope, thus favoring the escape of the hæmoglobin when a degree of internal tension has been reached which would still leave the normal corpuscle intact."[1]

BIOLOGICAL HÆMOLYSIS. Granting that the toxins of disease do cause a diminished resistance of the envelope, it is of interest to inquire whether at the same time an increased permeability of the envelope is produced. And if so, whether the corpuscle becomes more permeable to ions or to the hæmolytic agents only. It will be remembered that sapotoxin causes a primary increase in the permeability of the envelope, and by analogy one might reason that the intermediary body necessary to biological hæmolysis acts by making the envelope more permeable to the complement. In a conjoint experiment performed by Dr. Stewart and myself no evidence was obtained that dogs' serum can produce any change in the conductivity of the rabbit blood before liberation of hæmoglobin takes place.[2] This is also in agreement with the fact that the permeability of the formaldehyde-fixed corpuscles of the rabbit is not affected by dogs' serum. Since the envelope is not made more permeable to ions by the intermediary body, it is likely that the function of the latter is to effect such changes that the complement will be able to penetrate to the interior of the corpuscle, where it can exert its specific action.

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[1] Hamburger, Osmotischer, Druck und Ionenlehre, p. 391.

[2] American Journal of Physiology, No. 2, vol. ix. p. 81.

PROPERTIES OF THE ENVELOPE. The foregoing will, I think, be considered satisfactory evidence of an envelope in the red corpuscles, and we shall proceed to discuss the role that the envelope plays in hæmolysis and agglutination.

It will be appropriate at the outset to review what is known regarding the chemical and physical properties of the envelope. That it is hæmoglobin-free we have demonstrated by the action of hydroxylamine hydrochlorate and also by experiments with acid salts. (See previous pages.) That the envelope contains nucleoproteid as well as lecithin and cholesterin is shown respectively by the agglutination reaction with acids and acid salts and by the ether-laking experiments quoted previously. Alkalies must be present in combination with the nucleoproteid,[1] and salts, which always exist in protoplasm, must be contained in the envelope.

PHYSICAL PROPERTIES. The envelope of the red corpuscle appears to be smooth. That it also possesses a certain glaze I would infer from the crisp and sparkling reflex seen in fresh blood, and from the fact that the circulating corpuscles do not agglutinate with each other. This property of the envelope may subserve some defensive purpose. We can readily imagine that a corpuscle having a smooth surface will not offer so good a foothold—if the expression may be allowed—to any toxin, as would a corpuscle having a sticky envelope such as is produced by the action of an agglutinin. In support of this view we find that, as a rule, hæmolysis is preceded by agglutination. This is especially true of biological laking. In nature, wherever a biological hæmolysin occurs, there is usually also present an agglutinin in the same medium, and it seems as though the two bear a co-operative relation similar to that found between a complement and its intermediate body—which likewise are usually coexistent in nature.

When blood corpuscles are exposed to the simultaneous action of a hæmolysin and an agglutinin, the result varies with the relative strengths of the two. If the toxin be relatively weak, and the agglutinin strong, then we get primarily a powerful agglutination of the corpuscles followed by their gradual hæmolysis. If the toxin be very powerful and the agglutinin comparatively weak, then agglutination may not occur, especially if any swelling of the corpuscles is produced by the toxin. It seems that swelling of the corpuscles is inimical to agglutination.[2]

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[1] Compare Hammarsten, Physiolog. Chemie, p. 80.

[2] Peskind, American Journal of Physiology, No. 5, vol. viii. p. 416.

The envelope is distensible and elastic. It is this elasticity of the envelope which permits of the expansion and shrinkage of the corpuscle brought about by osmosis. Most hardening agents do not affect the elasticity of the envelope to any degree, since after the corpuscles are fixed by these reagents they still may be caused to swell.

The envelope being protoplasmic, and therefore semifluid, must exhibit the phenomenon of surface tension. This surface tension is one of the forces which co-operate to give the corpuscles their biconcave shape. During equilibrium the intraglobular pressure must be equal to the sum of the surface tension and the pressure exerted by the liquid about the corpuscle. If for any reason the surface tension be lessened, then the intraglobular pressure could assert itself and would tend to produce a greater distention of the envelope.

Such a diminution of the surface tension in blood corpuscles is brought about, I believe, by the emulsifiers, sapotoxin and sodium taurocholate, which are known for their ability to lessen the surface tension of liquids.[1] Reasons for this view will be advanced in the discussion of the permeability of blood corpuscles.

PERMEABILITY OF ENVELOPE. A very important property of the envelope is the selective permeability with which it is endowed. A great deal of investigation has been bestowed upon this complex subject, but it still rests upon a rather unsatisfactory basis. One fact has been pretty well established, namely, that the envelope is not permeable to salt molecules but to ions only. Not all ions, however, can penetrate the corpuscles; for the most part it is the electronegative ions, the halogens and acid radicals, which have this power. The envelope is permeable for NH_4 and to free acids and alkalies, but not for Ba, Str, Ca, Mg, and in all probability it is impermeable to Na and K, although this is still under some dispute.

Of the non-electrolytes there are a considerable number which can enter the corpuscles, among which may be mentioned alcohols, aldehydes, ketone, ether, antipyrin, urea, urethane. The corpuscles are impermeable to the various sugars, but allow bile acids and salts to penetrate.[2]

One can readily imagine that the size of certain molecules may be a cause of their inability to pass through the envelope. But if the intermolecular spaces of the envelope be enlarged—as must occur when the corpuscle swells and the envelope becomes distended—then it is conceivable that some large-sized molecules could enter which, under normal circumstances, would be unable to penetrate the corpuscles.

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[1] Hay's test for bile salts in urine is founded on the fact that these salts lower the surface tension of the urine.

[2] Hamburger, Osmotischer, Druck und Ionenlehre, p. 260.

Causing the corpuscle to swell is one way of enlarging the spaces which exist between the molecules of the envelope. Another method consists in removing some of the molecules which compose the envelope—*e. g.*, the cholesterin and lecithin. When these substances are extracted by ether the envelope is left in a porous condition, which makes it very permeable. This has been well shown in the experiment quoted above, in which formaldehyde corpuscles were extracted with ether. The conductivity of the corpuscles was found to be much increased by this procedure.

The suggestion may be ventured at this point that the action of sapotoxin consists partly in widening the intermolecular spaces of the envelope, making the latter more permeable to the sapotoxin molecules, which are presumably of large size. I conceive the mechanism of the process to be as follows:

First, as Ransom has shown, sapotoxin is taken up by the cholesterin of the envelope. The cholesterin and lecithin molecules are bound to the other molecules of the envelope merely by cohesion and not by firm chemical ties, since they may be dissolved out mechanically by ether. Now sapotoxin is an emulsifier, and, being such, lessens the cohesive power of fatty particles. Therefore, sapotoxin, once it has gained a foothold in the envelope, lessens the cohesion between the cholesterin-lecithin group and the other molecules which compose the protoplasm. Since surface tension is the result of the force of cohesion exerted between molecules of the surface layer, it follows that sapotoxin, in lessening the cohesion of these molecules, also lessens the surface tension of the corpuscles.

As soon as the surface tension becomes diminished the intraglobular pressure exerts itself and distends the envelope. Ions and water must now enter and the corpuscle swells.

This theoretical description corresponds very well to the actual first stage of sapotoxin laking. The corpuscles are seen to lose their biconcave shapes, become globular, and swell, showing that a great change in the surface tension has taken place, and that water and ions have entered.

At the same time it can be demonstrated that a great increase in the electric conductivity has taken place, which is probably due to the enlargement of the intermolecular spaces which accompanies the distention of the envelope.[1] The size of these interspaces being increased, the large-sized sapotoxin molecules can enter the corpuscles and split up the hæmoglobin-stroma compound, thus giving rise to laking.

DETERIORATION OF THE ENVELOPE. The red corpuscles, like other cells of the body, are often exposed to pathological influences and suffer changes which may seriously affect their value to the organism. Some of these alterations are without doubt due to a deterioration of the envelope and stroma, and it will be of interest in this connection to consider under what conditions the envelope of the red corpuscles will become damaged.

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[1] It may be noted here that sodium taurocholate causes laking without any notable previous swelling of the corpuscles; the increase in the permeability produced by this reagent is also much less than that produced by sapotoxin.—G. N. Stewart.

The following agents may be cited as producing deleterious changes in the envelope:

1. Toxins produced in disease.
2. Chemicals—*e. g.*, acids and alkalies.
3. Drying of corpuscles.
4. Fixing blood by throwing it into boiling saline.
5. Freezing and thawing.
6. Agglutinins.
7. Aging of corpuscles.

We shall discuss these in the order mentioned.

1. *Action of Toxins Produced in Disease.* The change in the resistance of the envelope produced by hæmolysins has been considered elsewhere. A diminished resistance of the corpuscles in typhoid, pneumonia, and other diseases has been constantly observed and is in all probability due to a deterioration of the envelope caused by the specific toxins formed during the course of these diseases. Some evidence to this effect may be derived from experiments that I recently made on the sera and corpuscles of various affections.[1]

An observation, which I have made on the blood of some severe cases of anæmia and several cancer cases may be mentioned here. On exposing a drop of blood to pressure between cover-slip and slide the hæmoglobin escapes quite readily from the corpuscles. (A similar phenomenon is observed in the case of corpuscles precipitated by ammonioferrous sulphate or other acid salts.)

Hamburger[2] has shown that pressure has no effect on the resistance of normal corpuscles, so that the above observation tends to show that the resistance of the envelope had been lowered by some product of the disease.

Vacuolization. I should like to suggest here that the condition known as "vacuolization of the hæmoglobin" might be due to a localized deterioration of the envelope. Let us imagine that a very small part of the circumference of a corpuscle were injured; this would allow the very gradual escape of hæmoglobin at this point and at the same time would permit water and ions to enter so that a seeming vacuole would be produced. (See Fig. 9.)

According to this view "vacuolization of the hæmoglobin" may be considered as a localized laking of the corpuscle.

2. *Action of Chemicals.* Acids and alkalies both effect the resistance of the envelope. Corpuscles acted upon by acids give up their hæmoglobin in a weaker NaCl solution than the normal corpuscles,

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[1] American Medicine, No. 23, vol. v. p. 919.

[2] Ioneulehre, p. 175.

while the contrary is the case with blood corpuscles acted upon by alkalies.[1]

3. *Drying of Corpuscles.* The effect produced on the envelopes of red corpuscles by allowing the latter to dry in air has been carefully studied by Dr. Guthrie.[2] He made the interesting observation that chickens, and human red blood corpuscles, allowed to dry in the air at room temperature, are readily laked by 0.9 per cent. NaCl solution.

It has been shown that fixing washed corpuscles by pouring them into boiling salt solution greatly increases their permeability to ions. Dr. Guthrie reasons "that a similar effect is produced by drying at a temperature below that necessary for fixation, so that the permeability of the corpuscles for diffusible substances is greatly increased, while it allows of the escape of hæmoglobin when enough water to dissolve it is present."

I have observed that when a drop of blood or of a washed suspension of corpuscles is placed on a slide and covered with a slip, those corpuscles which are at the edge of the drop—*i. e.*, where a gradual drying out occurs—become markedly swelled. And it may be demonstrated that there is also extensive laking at the edge of the drop, even in the case of corpuscles which at the moment of laking are floating in the liquid.

Dr. Guthrie in mentioning these experiments explains them very satisfactorily on the hypothesis "that the drying of a portion of the circumference of a corpuscle at the edge of the drop—or the action on it of the concentrated salt solution produced by evaporation —may render the rest of the circumference more freely permeable to the salts of the solution or to the water, and thus give rise to water laking."

4. Throwing blood corpuscles into boiling salt solution greatly increases their permeability to ions.[3]

5. Freezing and thawing causes laking, and while I have no proof to offer that this is due to an injury to the envelope, yet such an assumption seems very reasonable. The alternate contraction and expansion produced by freezing and thawing could very well have a disintegrating effect on a protoplasmic envelope consisting of heterogeneous molecules but loosely combined.

6. Agglutinins cause marked changes in the properties of the envelope, and that it also diminishes the resistance of the latter may be seen from the fact that an agglutinated mass of corpuscles loses hæmoglobin if exposed to slight pressure between slide and coverslip. Normal corpuscles cannot be laked in this manner.

7. *Aging of Corpuscles.* Old corpuscles may be distinguished in the circulating blood by the fact that they have lost a large part of

[1] Ioneulehre, p. 322.

[2] American Journal of Physiology, No. 5, vol. viii. p. 444.

[3] G. N. Stewart.

their hæmoglobin. This is most likely due to the deterioration of the envelope effected by the wear and tear to which the corpuscles are exposed.

Soon after blood is shed from an artery its permeability to NH_4Cl is greatly increased.[1] This indicates that there is a difference between corpuscles within the blood stream and those *in vitro*. It may be shown, moreover, that the envelopes of corpuscles which have been removed from the body deteriorate after a certain length of time. The behavior of corpuscles to hydroxylamine hydrochlorate may be cited as proof of this. Fresh blood, and blood less than three days old, when treated with this reagent, show bubble-formation within the corpuscles (see above). Blood that has stood for about three days will not show any bubbles with the reagent, but the corpuscles have a perforated appearance as though gas bubbles had escaped from their interior. This may be explained as follows:

Hydroxylamine hydrochlorate has a double action; not only does it permeate the corpuscles and produce gas bubbles within them, but it hardens them at the same time. If the envelope be intact, then the reagent penetrates but slowly and thus has time to harden the envelope before much of the salt enters the corpuscle. The evolution of gas produced by the reagent is thus very gradual and, as the bubbles are formed at the periphery of the corpuscle, they are retained by the partly hardened envelope. When the blood becomes old, however, the envelope deteriorates, in consequence of which the hydroxylamine hydrochlorate penetrates very rapidly, before the envelope is hardened sufficiently to be able to retain the bubbles. The result is that the reagent which enters the corpuscles in large quantities gives rise to an immediate and copious evolution of gas bubbles whose escape cannot be hindered by the weakened and only slightly hardened envelope.

The rents in the envelope produced by the escaping bubbles remain open and give rise to the perforated appearance above mentioned.

This observation shows strikingly how the envelope deteriorates with the aging of the blood corpuscles.

HARDENING OF ENVELOPE. The effects of various hardening agents on the envelope and stroma are of considerable importance and merit some mention here. Among the principal hardening and fixing agents may be mentioned $HgCl_2$ (Hayem's solution), formaldehyde, osmic acid, hydroxylamine hydrochlorate, alcohol, ether, chromic acid.

In another part of the paper was brought out the fact that the distensibility and elasticity of the envelope and stroma were practically unaltered by most hardening agents, since the hardened corpuscles may still be caused to swell by suitable means.

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[1] G. N. Stewart.

Agglutination of corpuscles hardened by formaldehyde, osmic acid, and ether, may still be brought about by certain means. But after fixation with $HgCl_2$ I was unable to produce agglutination. Chromic acid and picric acid agglutinate while they harden the corpuscles.

The envelopes of corpuscles hardened by formaldehyde and those hardened by $HgCl_2$ show a dissimilarity in their behavior toward sapotoxin; whereas the electric conductivity of formaldehyde-hardened corpuscles is greatly increased by sapotoxin, corpuscles hardened by $HgCl_2$ show no such effect, presumably owing to the more complete hardening produced by the latter reagent.

A peculiar effect is produced by acid salts when added to blood just shed from an artery. If to a definite amount of blood taken from the carotid and diluted with saline is added just enough ferric chloride to precipitate all the corpuscles, it will be found that the supernatant serum, after the corpuscles have settled, will not clot even after three days. This serum is neutral in reaction. The ferric chloride, combining as it does with the envelopes of the corpuscles, probably prevents the exit of the fibrin ferment, but possibly the neutralization of the alkali in the serum by the ferric chloride is the cause of its not clotting. The former mode of action is the most likely, since the addition of alkali to the serum did not produce real clotting. Further experiment must decide this point.[1]

RESUME. Various facts of a histological, chemical, and physical character show that the red blood corpuscles possess an envelope.

From the action of hydroxylamine hydrochlorate, which produces sharply circumscribed accumulations of nitrogen gas—*i. e.*, bubbles —on the periphery of blood corpuscles and not an extensive separation of the envelope from the contents underneath, it appears most probable that the envelope is not a differentiated membrane, but a part of the stroma which is condensed to form the surface layer of the corpuscle.

The envelope is hæmoglobin-free and consists of nucleoproteid, cholesterin, lecithin, and mineral constituents.

It is elastic, smooth, and apparently possesses a certain glaze which prevents the agglutination of normal corpuscles with each other and makes the corpuscles less accessible to the action of toxins.

Agglutination of blood corpuscles is due to an effect on the envelope produced by various biological products and chemical reagents whereby the envelope is made sticky.

Agglutinins, by modifying the envelope, probably lower the resistance of blood corpuscles toward toxins and other agents. From the fact that in nature they almost always occur in company with a hæmolysin, the suggestion is offered that the agglutinins bear some co-operative relation to the hæmolysins similar to that existing between the intermediary body and the complement, which likewise are usually coexistent in nature.

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[1] American Journal of Physiology, 1903, vol. viii. p. 412.

The "resistance" of blood corpuscles depends in large part upon the condition of the envelope. The latter may be caused to deteriorate by various agents principal among which are the toxins of disease. Deterioration of the envelope may be complete or partial —*i. e.*, involving the whole circumference or only a small part of the same.

So-called "vacuolization of the hæmoglobin" can be explained very satisfactorily on the assumption of a minute lesion in the envelope which allows the surrounding fluid to enter and thus permits of a localized laking at this point.

The function of the envelope is in part to make possible various metabolic processes, principal among which is the complex process known as internal respiration. This is accomplished through the medium of a selective permeability.

Another important use of the envelope is to protect the corpuscles from the action of various substances deleterious to the latter. But the very chemical constitution of the envelope may at times serve for the undoing of the corpuscles, since it has been well shown by Ransom, Kyes, Sachs, and others that the cholesterin and lecithin of the envelope fix certain toxins and thus act as intermediary bodies.

HÆMOLYSINS IN HUMAN URINE.*

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Since the epoch-making experiments of Bordet,[1] such a vast amount of work has been done upon hæmolysis and allied subjects that a review of the literature seems superfluous. For present purposes, it is only necessary to refer to some of those instances in which specific bodies have been shown to pass from the blood into the secretions or excretions.

Ehrlich and Brieger[2] showed, in the case of a goat immunized against tetanus toxin, that the specific antibody could be demonstrated in the milk. By Kassel and Mann[3] it was shown that the milk of a woman suffering from typhoid fever possessed the power of agglutinating typhoid bacilli.

Friedberger[4] was the first to demonstrate the passage of agglutinins and hæmolysins from the blood into the urine. For this purpose

* Read at the meeting of the American Association of Pathologists and Bacteriologists at New York, April 1 and 2, 1904.